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A new genus and species of nicoletiid silverfish (Insecta, Zygentoma, Nicoletiidae) from caves of northern Alabama, USA

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Abstract

A new genus and species of troglobiotic nicoletiid (Insecta, Zygentoma, Nicoletiidae) is described from northern Alabama, USA. The type species was collected from three caves in the Highland Rim section of the Interior Low Plateau physiographic province on the northern side of the Tennessee River Valley. Morphological and genetic analysis using the mitochondrial 16S rRNA locus show that *Spinanycta alabamensis* **sp. nov.** is quite distinct from related nicoletiids in North America. The species differs from members of other genera by its urosternum I, which in males is modified with a central pointy extension. The new species significantly extends the distribution of cave-dwelling members of the family into the southeastern United States and suggests that additional nicoletiid diversity remains to be discovered from karst regions of the eastern United States.

Keywords

Insect, Interior Low Plateau, subterranean, Thysanura, troglobite, troglobiont

Introduction

Among the insects, Silverfish (order Zygentoma) are some of the most intriguing. This group diverged before the appearance of wings and thus are considered among the earliest, most primitive insects. Their predecessors were among the first arthropods to colo-

nize terrestrial habitats and were present during the Silurian Period more than 400 million years ago (Grimaldi and Engel 2005). Within Zygentoma, members of Nicoletiidae typically live in habitats lacking light, with distinctive characteristics for a subterranean existence, such as lack of pigment and eyes. While intrinsically interesting because of their specialized ecology, our understanding of their biodiversity has been greatly constrained, like many troglobionts (Niemiller et al. 2018; Mammola et al. 2019; Niemiller and Taylor 2019), by the difficulties inherent in collecting samples from subterranean environments. For example, the subfamily Cubacubaninae is a seldom encountered group of silverfish that were considered a minor group of little importance primarily restricted to the Neotropics, where they are found under rocks and logs (Espinasa et al. 2007). However, Cubacubaninae is now known to be a much more diverse group with a range extending into southern North America owing to recent studies by cave biologists (Espinasa et al. 2014; Fig. 1). Members of the genus *Texoreddellia* Wygodzinsky, 1973 have been described and documented from more than 150 caves in Texas, USA, and northern Mexico (Espinasa et al. 2016, 2023). *Speleonycta ozarkensis* Espinasa et al., 2010 is known from eight caves in the Ozarks Highlands region of Oklahoma and Arkansas (Espinasa et al. 2014; M.E. Slay, personal communication). *Speleonycta anachoretes* Espinasa et al., 2012 is known from two caves in Arizona, and three caves from California are inhabited by an undescribed species of *Speleonycta* Espinasa et al., 2010.

Here we describe a new genus and species of cave nicoletiid from caves of the Interior Low Plateau karst region in northern Alabama, recently discovered during cave biosurveys in the region. The new species significantly extends the distribution of cave-dwelling members of the family into the southeastern United States and increases the likelihood that additional diversity remains to be discovered in the Interior Low Plateau and other karst regions of eastern North America.

Material and methods

Specimen collection and approach

Specimens were collected as part of a biological inventory of cave life in northern Alabama. Biosurveys consisted of visual encounter surveys (VESs) by a minimum of two people for at least two-person hours in all caves. VESs of terrestrial habitats included the cave floor, walls, and ceiling, as well as underneath rocks, logs, and other debris when available. Any cover objects were returned to their original positions to minimize microhabitat disturbance. Specimens encountered were collected by hand and preserved in 100% ethanol, then stored at -20 °C for molecular analysis. Specimens were accessioned into the Entomology Collection at the Auburn Museum of Natural History.

Morphological analyses

Specimens were examined and dissected using a Motic stereomicroscope. Photographs of some structures were recorded using an iPhone 12 mini camera attached to the dis-

secting microscope. Photographs were taken in multiple focal planes and processed so that the whole image was in focus using the Zerene Stacker software package (Zerene Systems LLC, Richland, Washington, USA). Drawings of some pertinent structures were prepared with the aid of camera lucida attached to a Motic dissecting microscope.

DNA extraction, PCR amplification, and sequencing

Genomic DNA was extracted using Qiagen's DNEasy® Tissue Kit by digesting a leg in lysis buffer from five specimens from Muddy Cave in Madison County, Alabama (Alabama Cave Survey (ACS) cave no. AMD1095) collected on 7 July 2022, one from Rockhouse Cave in Limestone County, Alabama (ACS no. ALM312) collected on 21 May 2020, and the holotype and three paratypes from Bobcat Cave in Madison County, Alabama (ACS no. AMD1283) collected on 26 November 2019.

PCR amplification and Sanger sequencing of a portion of the mitochondrial 16S rRNA locus followed standard protocols and primers (*16Sar* and *16Sb*) used in the past for nicoletiids (Espinasa and Giribet 2009). PCR products were purified using QIAquick® PCR Purification Kit and sequenced by Elim Biopharmaceuticals Inc (Hayward, California, USA). Resulting sequences were visualized and assembled into contigs using the sequence editing software Sequencher™ 3.0. Sequencing primers were trimmed and not included in subsequent analyses.

Phylogenetic analyses

BLAST analysis was done to determine the most similar sequences in GenBank. Sequences of all other nicoletiid species available were aligned and a preliminary tree analysis was performed in ClustalW2 (ML tree was inferred using PhyML v20160115 ran with model and parameters: -o tlr -f m --pinv e --bootstrap -2 --alpha e --nclasses 4. Branch supports are the Chi2-based parametric values return by the approximate likelihood ratio test). DNA sequences for specimens are in NCBI's GenBank (accession no. PP057689).

Conservation assessment

We conducted both NatureServe and IUCN Red List conservation assessments for the newly described species. For the IUCN Red List assessment, the risk category was calculated using RAMAS Red List 3.0 (Akcakaya et al. 2007) following criteria in IUCN (2010, 2012). For the NatureServe assessment, we employed the NatureServe Rank Calculator v3.186 (Faber-Langendoen et al. 2012) following criteria in Master et al. (2009). We calculated two different measures of geographic range size using the web-based program GeoCAT (Bachman et al. 2011; available at https://geocat.kew.org): extent of occurrence (EOO) as a minimum convex hull and area of occupancy (AOO) using a grid size of 2 km (4 km^2). Finally, we employed the threat classification scheme proposed by Salafsky et al. (2008) to determine the overall threat impact to the species.

Results

Class Insecta Linnaeus, 1758
Order Zygentoma Börner, 1904
Suborder Neozygentoma Engel, 2006
Infraorder Euzygentoma Grimaldi & Engel, 2005
Family Nicoletiidae Escherich, 1905

***Spinanycta* Espinasa, Gutierrez & Niemiller, gen. nov.**
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Diagnosis. An American nicoletiid with Urosterna II–VII subdivided into two coxites and one sternite. Urosterna I, VIII and IX of male entire. Coxites on segments II–IX with styli. Urosternum I of males modified with a central pointy extension. Urosternum VIII of male flat posteriorly, without emarginations or projections in between the styli of this segment. Paramera with a distal semi-eversible vesicle and short chaetae, their length being about 1/4 the width of the paramera. Subgenital plate of females subtriangular. Tergum X with one distinct macrochaetae on posterior angles. Cercus of male with sensory pegs (spines) and appendix dorsalis without sensory pegs.

Description. Pedicellus of adult male with unicellular glands. Mouthparts not specialized. Mandible strongly sclerotized apically with usual teeth. Apex of galea with two conules; one longer than wide and the other wider than long. Lacinia heavily sclerotized distally. First process of lacinia pectinate. Labium without prominent lateral lobes.

Tarsi with four articles. Praetarsi with three simple claws. Median claw glabrous, slender and smaller than lateral claws. Urosterna II–VII subdivided into two coxites and one sternite. Urosterna VIII and IX of male entire. Median portion of sternites with 1+1 sublateral macrochaetae at hind borders, as well as 1+1 macrochoetae near suture at about middle of segment. Coxites on segments II–IX with styli. Eversible vesicles on segments II–VI, pseudovesicles on VII. Urosterna I of males modified with a central pointy extension. Urosterna III and IV of adult males apparently without modifications. Urosternum VIII of male straight posteriorly, without emarginations or projections in between the styli of this segment. Tergum X with 1+1 distinct macrochaetae on posterior angles.

Point of insertion of paramera apparently slightly deep. Paramera with a distal semi-eversible vesicle, but less prominent than in *Texoreddellia* (Wygodzinsky 1973) or *Speleonycta* (Espinasa et al. 2010) and with distal chaetae short, their length being about 1/4 the width of the paramera. Stylus IX apparently without sensory pegs in males as seen in some species *Prosthecina* Silvestri, 1933 or *Anelpistina* Silvestri, 1905 (= *Cubacubana* Wygodzinsky & Hollinger, 1977; syn. = *Neonicoletia* Paclt, 1979) (Espinasa et al. 2007). Opening of penis longitudinal. Cercus of male with sensory pegs. Appendix dorsalis without sensory pegs. Female with a subtriangular subgenital plate.

Molecular diagnosis. Sequences generated for the mitochondrial 16S rRNA locus for all ten specimens from the three different caves were 529 bp long and identical.

BLAST analysis showed this 16S haplotype to be quite different from any sequence available in GenBank (maximum of 77.26 Per. ident.). Within the American Nicoletiidae, *Spinanycta* differs from the genus *Allonicoletia* Mendes, 1992 by 130 bp (24.5%), from *Speleonycta* by 132 bp (24.9%), from *Texoreddellia* by 134 bp (25.3%), from *Squamigera* Espinasa, 1999 by 138 (26.0%), from *Gibboletia* Espinasa & Smith, 2023 by 145 bp (27.4%), from *Prosthecina* by 148 bp (27.9%), from *Anelpistina* by 155 (29.3%), and from *Nicoletia* Gervais, 1843 by 153 bp (28.9%). Using the 16S rRNA fragment sequences of nicoletiid species across the subfamily Cubacubaninae (Espinasa et al. 2007), it can be observed that pairs of specimens from different populations of the same species differ by an average of 3.4 nucleotides (range 0 to 13; n = 22) and by 31.2 nucleotides (range 10 to 64; n = 14) among sister species. The 130+ bp between the Alabama specimens and any previously described species of American nicoletiid suggests these populations represent a new species and genus.

Maximum-likelihood phylogenetic analysis showed that members of genera *Speleonycta*, *Allonicoletia*, *Texoreddellia* and *Nicoletia* form a well-supported clade, with the Alabama specimens as sister to this group, and also in a clade distinct from other nicoletiid genera including *Anelpistina*. These results indicate that the Alabama nicoletiids belong to a group independent from any previously described genera for which 16S rRNA sequences are available. *Acanthonima* Espinasa, 2005 has yet to have its DNA sequenced, but morphologically it is very different from the Alabama specimens (See below). Resolving the placement of all aforementioned genera within subfamilies was beyond the goals of the current study.

Type species. *Spinanycta alabamensis* sp. nov.

Etymology. *Spinanycta*. From *spina* = Greek for spine. It references the diagnostic spine in urosterum I, and *nycta* = Greek for night. It references in Greek mythology the occupation of caves by Nyx, the primordial goddess of night.

Remarks. *Spinanycta alabamensis* belongs to the American Nicoletiidae characterized by subdivided abdominal sterna II–VII and fused coxites of abdominal segments VIII and IX (Cubacubaninae + Nicoletiinae; Mendes 1988), or to a still undescribed subfamily that shares these characteristics. *Spinanycta alabamensis* is distinguished from all genera of these subfamilies by the urosterna I of males modified with a central pointy extension, although male sexual secondary characters in urosterna I–IV are highly variable among species.

The type species of the new genus shares some characteristics with *Speleonycta*, *Allonicoletia*, *Texoreddellia*, and *Nicoletia*, such as the paramera with semi-eversible vesicles, urosternum VIII of male straight posteriorly without emarginations or projections in between the styli of this segment. None of these characteristics are present in any of the other genera of Cubacubaninae. The new genus can easily be distinguished from *Speleonycta* because it lacks the very long and specialized chaetae in the distal semi-eversible vesicle of the paramera. From *Allonicoletia* they can further be differentiated by the presence of styli on urosternite II. From *Texoreddellia* and *Squamigera* by the absence of scales. From *Prosthecina* by the absence of conspicuous lateral lobes bearing numerous glandular pores in the submentum. From *Anelpistina* as defined by

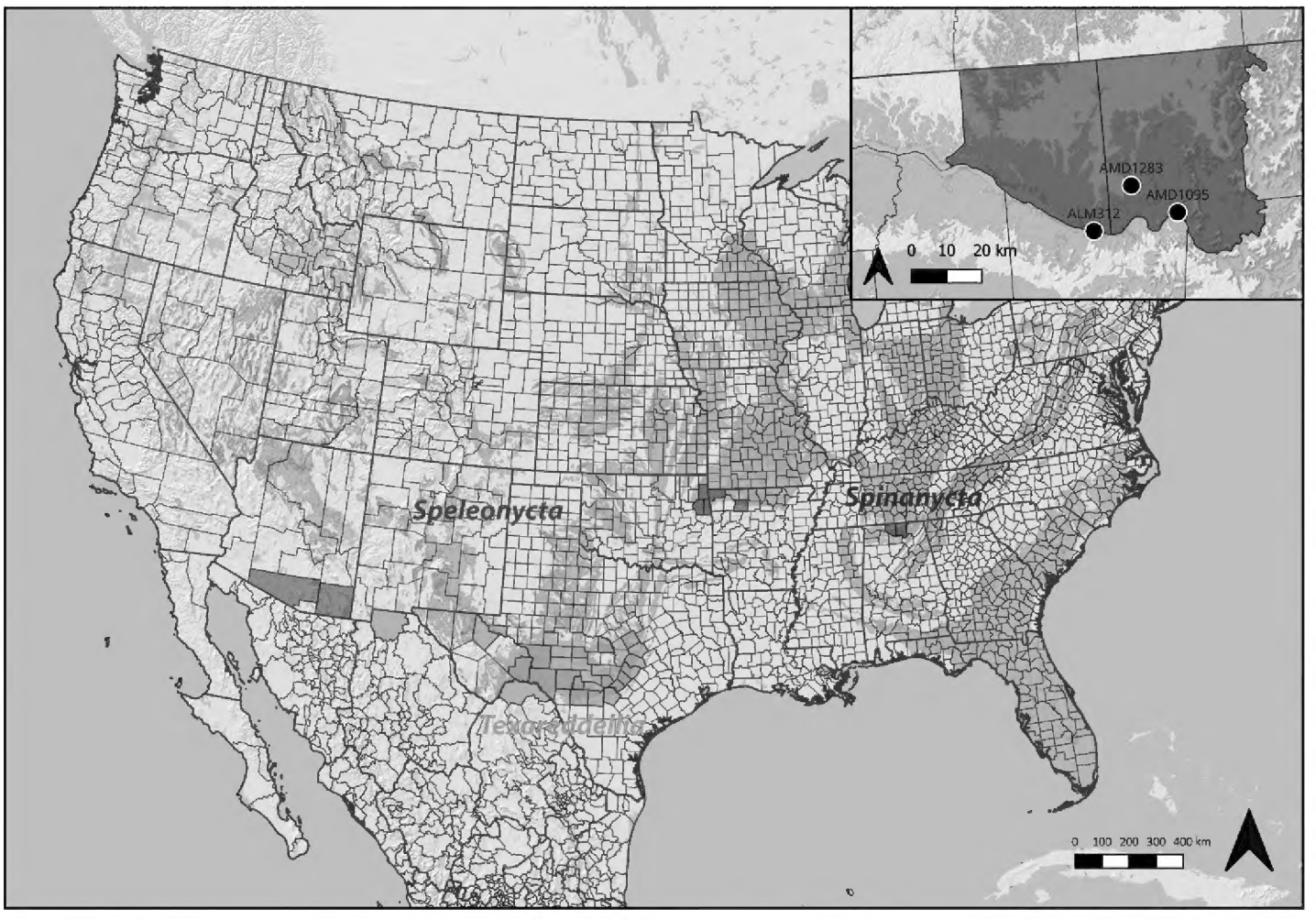

Figure 1. Distribution of cave-dwelling nicoletiid genera in northern Mexico and the southern United States. Shaded areas are municipalities and counties in Mexico and the United States, respectively, with documented occurrences for each genus. Dark gray shaded areas are karst exposures in the United States. Inset is the distribution of *Spinanycta alabamensis* sp. nov. in northern Alabama with Alabama Cave Survey cave codes labeled. Bobcat Cave (AMD1283) is the type locality.

Espinasa et al. (2007), by its urostenum VIII without emarginations or projections in between the styli of this segment and its distinctive paramera. From *Acanthonima* by the absence of sensory pegs in the appendix dorsalis. From *Nicoletia* by its gonapophysis with more than 12 annuli.

Distribution. This genus is known from just three cave systems on the north side of the Tennessee River Valley in southern Madison and southeastern Limestone counties, Alabama, within the Highland Rim section of the Interior Low Plateau physiographic province (Fig. 1).

Spinanycta alabamensis Espinasa, Gutierrez & Niemiller, sp. nov.

https://zoobank.org/A7588D17-B2DA-4B00-839B-6E49C30CF3D6
Figs 2–4

Type material. ***Holotype***: male 8.5 mm, from Bobcat Cave (34.6686°N, 86.7130°W), Alabama Cave Survey (ACS) no. AMD1283. Redstone Arsenal, Madison County, Alabama, USA, deposited into the Entomology Collection at the Auburn Museum of Natural History (AUM 221500), collected 26 November 2019 by Matthew L. Niemiller, Joseph Lamb, Amata Hinkle, and Brian Stoltz (field code MLN 19-050).

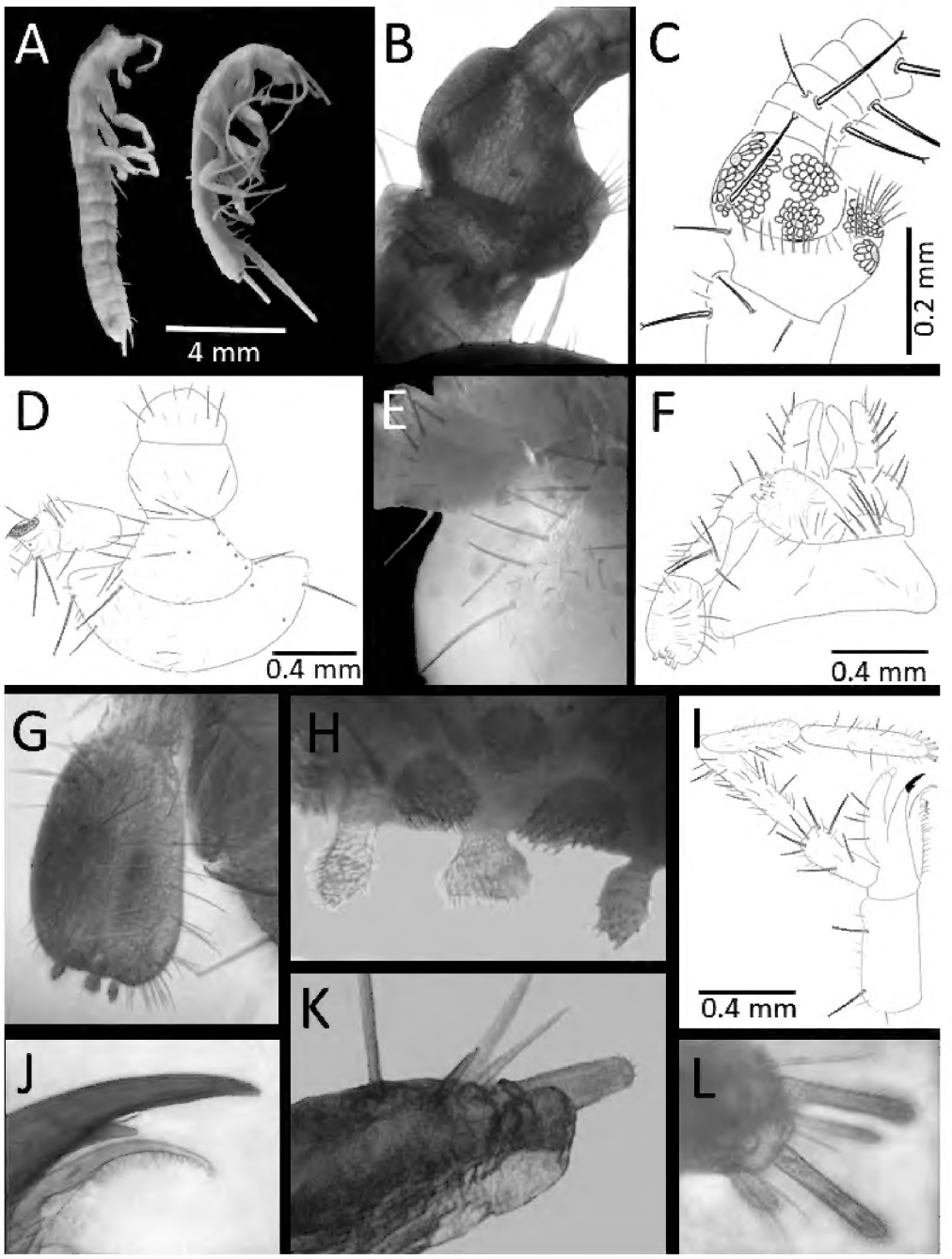

Figure 2. *Spinanycta alabamensis* sp. nov. **A** male holotype (left) and female paratype (right) **B–L** male holotype **B, C** pedicellus **D** head **E** macrochaetae by insertion of antennae **F** labium **G** last article of labial palp **H** sensilla of maxillary palp **I** maxilla **J** apex of lacinia **K** conules of galea **L** sensilla of maxillary palp.

Paratypes: 3♀ paratypes with same collection information as holotype (AUM 221501–221503); 1♂, 3♀ paratypes from Bobcat Cave collected on 6 October 2020 by Matthew L. Niemiller, Joseph Lamb, and Amata Hinkle (field code MLN 20-036.6) (AUM 221504–221507); and 1♂, 1♀ from Bobcat Cave collected 13 September 2023 by Amata Hinkle, Brendan T. Cramphorn, Jared P. Higgs, and Eric C. Maxwell (field code BTC 23-019-1) (AUM 221508–221509).

Additional material: 2♂, 2♀ from Muddy Cave (ACS no. AMD1095), Madison County, Alabama, USA, collected 15 June 2020 by Matthew L. Niemiller, K. Denise Kendall Niemiller, Amata Hinkle, Joseph Lamb, and Katherine E. Dooley (field code MLN 2020-021.1) (AUM 221510–221513); 1♂, 2♀ from Muddy Cave collected 7 July 2022 by Matthew L. Niemiller, Brendan T. Cramphorn, and Eric Cline (field code BTC 22-110-3) (AUM 221514–221516).

1♀ from Rockhouse Cave (ACS no. ALM312), Wheeler National Wildlife Refuge, Limestone County, Alabama, USA collected 21 May 2020 by Matthew L. Nie-

miller, K. Denise Kendall Niemiller, Amata Hinkle, and Katherine E. Dooley (field code MLN 20-020.6) (AUM 221517).

Etymology. *S. alabamensis*. Derived from the word Alabama, the state where the species was discovered.

Type locality. USA. Alabama, Madison County, Bobcat Cave (ACS no. AMD1283) on Redstone Arsenal. Redstone Arsenal is a 154.8 km^2 United States Army installation in southwestern Madison County adjacent to the city of Huntsville. Redstone Arsenal is host to more than 75 tenant agencies, including the Department of Defense, Department of Justice, Federal Bureau of Investigation, and, most notably, the National Aeronautics and Space Administration's Marshall Space Flight Center. Bobcat Cave is located near the western border of Redstone Arsenal (34.6686°N, 86.7130°W) on a small knoll. Redstone Arsenal and Bobcat Cave are situated within the Tennessee River District of the Highland Rim section of the Interior Low Plateau Province. The cave is developed in the Tuscumbia Limestone with a mapped length of 454.1 m (1,490 ft) and vertical extent of 6.4 m (21 ft). Much of the cave consists of a series of low and broad passages connected by tight crawlways with little exposure of bedrock. Water levels in the cave respond quickly to heavy precipitation and can flood completely. Significant flooding events continually deposit mud on nearly all surfaces in the cave. It is on these mud substrates and under the few mud-encased rocks nearer the entrance that most *Spinanycta alabamensis* have been found. In addition, individuals have been observed on the surface of isolated pools in the Shrimp Room, the largest room in the cave (61 m by 30.5 m).

Description. Male holotype 8.5 mm (Fig. 2A) from Bobcat Cave, collected 26 November 2019. Thorax width 1.5 mm. When complete, antennae about 1.25× the length of the body and tails about 0.75×. General color whitish, yellow (Fig. 2A). When complete, antennae are about 1.25× the length of the body and tails about 0.75× the length of the body. Pedicellus about half the length of the scape, with unicellular glands clustered ventrally in four groups with a row of microchaetae bordering them in form of a "U". On its lateral outside side there are an extra two groups of unicellular glands in a large, bulbous projection (Fig. 2B, C). Female pedicellus simple and slightly less than half the length of the scape. Head with about 6+6 macrochaetae near the insertion of each antenna (Fig. 2D, E).

Mouthparts as shown in Figs 2F–L, 3A. Appendages longer than epigeomorphic species, but shorter than highly troglomorphic nicoletiids. Labial palp's apical article's long. Its width is about 2/3 its length and slightly longer than the penultimate article (Fig. 2F–G). As in other cave-adapted nicoletiids with long appendages, the bulge containing 2 macrochaetae in the penultimate article of the labial palp is not too prominent (Fig. 2F). Labium and first article of labial palp with macrochaetae. The sensilla of the labial palp are longer than other American nicoletiids due to being supported by a longer than common trunk (Fig. 2H). Last article of maxillary palp 1/3 longer than the penultimate article (Fig. 2I). Apex of lacinia as in Fig. 2J. Apex of galea with two conules; one longer than wide and the other wider than long (Fig. 2K). Apex of maxillary palp as in Fig. 2L. Mandible chaetotaxy with 4 macrochaetae (Fig. 3A).

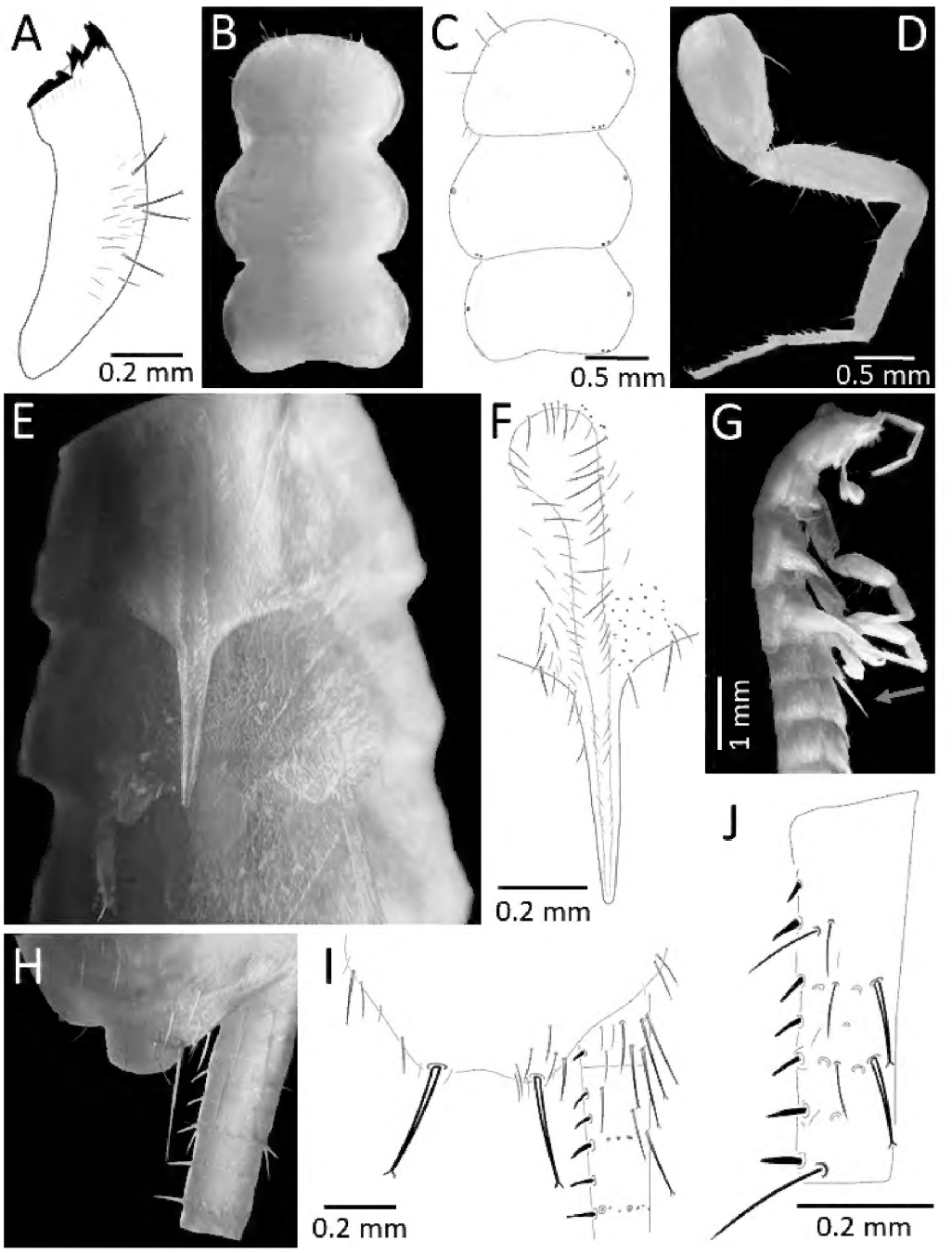

Figure 3. *Spinanycta alabamensis* sp. nov. **A–C, E–J** male holotype **D** female paratype **A** Mandible **B, C** thorax **D** hind leg **E, F** ventral view of spine of urosternum I **G** lateral view of male holotype highlighting (red arrow) the spine of urosternum I **H, I** urosterguite X **J** cercus.

Thorax as in Fig. 3B, C, with 1+1 macrochaetae on their lateral sides plus several posterolateral. Legs not too long when compared to other troglobitic nicoletiids, as shown in Fig. 3D. Hind tibia approximately 5 times longer than wide and approximately 3/4 length of tarsus. Claws of the praetarsus and empodium with a hairy appearance covered basally with small microtrichiae, similar to Molero-Baltanás et al. (2020; fig. 23). Abdominal sterna II–VII subdivided into coxites and sternites (Fig. 3E). Sterna I, VIII and IX of male entire. Styli on sterna II–IX. Sterna I of males modified with a long, narrow point extending to the apex of sterna II (Fig. 3E–G). This point has in its center a groove surrounded by many macrochaetae that extends to the base of sterna I (Fig. 3F). No apparent modifications in male's sterna III and IV.

Posterior end of urotergite X with a 1+1 long macrochaetae (Fig. 3H, I), similar to *Nicoletia phytophila* Gervais, 1844 (Espinasa et al. 2011; Fig. 1C, D). Distance between macrochaetae about the same as the length of the macrochaetae. Area between

the distinct macrochaetae with a small concavity (Fig. 3I). Cercus of adult male with a longer than wide basal annulus with one small spine, followed by a longer annulus, with multiple subequal spines (Fig. 3I, J). Female cerci without modifications.

Urosternum VIII of adult male flat between the insertion of the styli (Fig. 4A), without an emargination bordered by projections, as in other Cubacubaninae. Point of insertion of parameres in urosternum IX deep and with coxal processes with several setae slightly more sclerotized (Fig. 4A, B). Parameres as in Fig. 4A, B. Parameres barely surpass the base of styli. Paramera with a distal semi-eversible vesicle and with some short, modified setae (Fig. 4B). Stylus IX with 2 macrochaetae and an extra subapical pair, and larger than other styli (Fig. 4A). Other styli with 1 macrochaetae plus subapical pair. Terminal spine with one small tooth.

In adult females, the ovipositor surpasses apex of stylus IX between 3 and 3+2/3 the length of the stylus IX (Fig. 4G). Gonapophyses with about 21–22 divisions. The subgenital plate has a subtriangular shape (Fig. 4E, F) that ends in a slight point (Fig. 4G), similar to the one in *Nicoletia phytophila* (Espinasa et al. 2011; Fig. 1A, E), although the slight point is less prominent in the new species.

Postembryonic development. Males at 5.5 mm have no glands in pedicellus, no spine in urosternum I, or spines in cerci, but they already have paramers. At 6.5 mm they have glands in the pedicellus, the spine in urosternum I reaches about ¾ of the apex of urosternum II, and spines in cerci are small. At 7 mm the spine reaches the apex of urosternum II and by 8.5 mm it surpasses by 1/10 the length urosternum II.

Females at 5.5 mm have an ovipositor that barely surpasses the apex of stylus IX and gonapophyses with about 15 divisions. By 7 mm ovipositor surpasses apex of stylus IX by about 2+2/3 the length of the stylus and the gonapophyses has the adult number of 21–22 divisions. Between 7.2 mm and 10.5 mm in the largest available specimen the ovipositor surpasses stylus IX by between 3 and 3+2/3 the length of the stylus.

Geographic distribution. The species is known to date only from three caves, all developed in the Tuscumbia Limestone and located along the northside of the Tennessee River in southern Madison and southeastern Limestone counties. The largest population at Bobcat Cave is located on Redstone Arsenal. Muddy Cave is owned and managed by the Land Trust of North Alabama and is located 15.1 km SE of Bobcat Cave adjacent to a large residential area. Rockhouse Cave is located 16.5 km SW of Bobcat Cave and is developed in an exposure of limestone, Rock House Bluffs, on the north shore of the Tennessee River on Wheeler National Wildlife Refuge. The current distribution represents an EOO of 124.1 km^2 and AOO of 12.0 km^2.

Habitat and ecology. At Bobcat Cave, *Spinanycta alabamensis* has been observed actively crawling on mudbanks and underneath mud-covered cobble and rocks adjacent to pools in the dark zone below the slope from the entrance and throughout the Shrimp Room (Figs 5, 6). On two occasions, individuals were observed on the surface of a pool. As many as 20 individuals have been observed during a survey at Bobcat Cave (Table 1). At Muddy Cave, individuals have been observed on mudbanks and underneath mud-covered cobble near pools primarily before the tight crawl to the northern section of the 348-m (1143-ft) cave system. A single individual has been

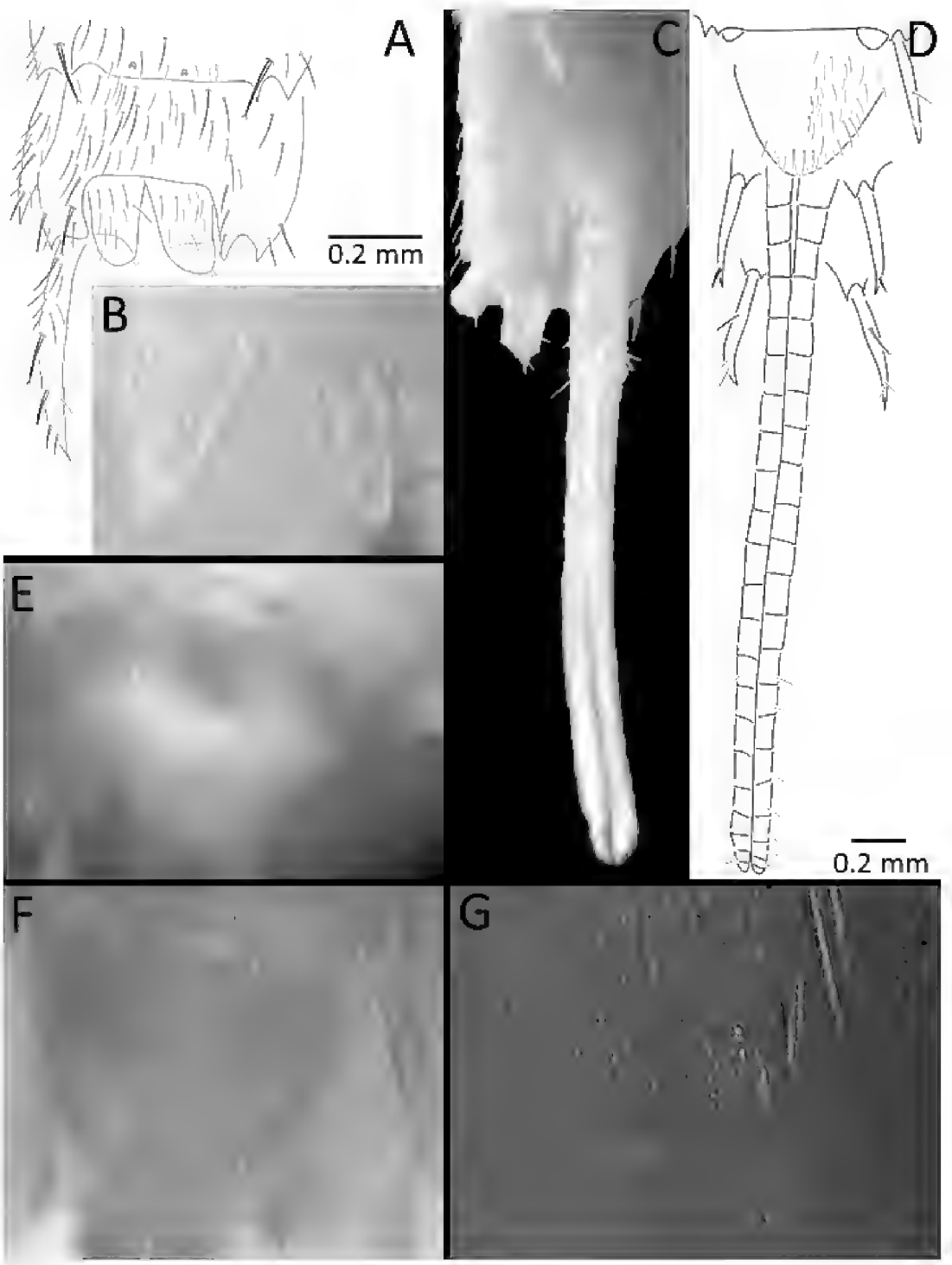

Figure 4. *Spinanycta alabamensis* sp. nov., **A, B** male paratype **C–G** female paratypes **A** male genital area **B** parameres highlighting (red arrow) distal semi-eversible vesicle **C, D** ovipositor **E, F** variability in the subtriangular shape of the subgenital plate **G** apex of subgenital plate highlighting (red arrow) its slight point.

observed and collected at Rockhouse Cave. A female was found under a small (12-cm diameter), loose, but mud-covered rock adjacent to an isolated pool in a fissure in the northern section of the 384-m (1259-ft) long cave system. All observations to date have been made in sections of the three cave systems that completely flood during heavy precipitation events. *Spinanycta alabamensis* co-occurs with several other troglobiotic taxa, including the spiders *Phanetta subterranea* (Emerton, 1875) and *Liocranoides unicolor* Keyserling, 1881, millipede *Scoterpes* sp. Cope, 1872, springtail *Pseudosinella* sp. Schaeffer, 1897, two-pronged bristletail *Litocampa* sp. Silvestri, 1933, and isopod *Miktoniscus* sp. Kesselyak, 1930.

Conservation. *Spinanycta alabamensis* is known currently from three caves in southern Madison and southeastern Limestone counties, Alabama. The species was

Table 1. Observations of *Spinanycta alabamensis* sp. nov. from the three known localities in northern Alabama, USA.

	Date	No. observed
Bobcat Cave (AMD1283), Madison Co., Alabama	26 Nov 2019	7
	6 Oct 2020	5
	13 Sep 2023	20
Muddy Cave (AMD1095), Madison Co., Alabama	15 Jun 2020	13
	7 July 2022	4
	25 Sep 2022	1
	27 Aug 2023	0
Rockhouse Cave (ALM312) Limestone Co., Alabama	21 May 2020	1
	24 Nov 2021	0
	3 April 2022	0

Figure 5. *Spinanycta alabamensis* sp. nov. female in life, observed on 13 September 2023 at the type locality Bobcat Cave, Madison County, Alabama, USA. Photograph by Eric C. Maxwell.

assessed as Imperiled (G2) under NatureServe criteria and as Endangered B1a under IUCN Red List criteria because of few known occurrences, a small and potentially restricted geographic range, high intrinsic vulnerability, and several current and potential threats to populations. All three caves occur on protected lands: Bobcat Cave on Redstone Arsenal, Rockhouse Cave on Wheeler National Wildlife Refuge, and Muddy Cave is owned by the Land Trust of North Alabama. Populations face several existing and potential threats. Pollution from surface runoff associated with continued urbanization in the Huntsville metropolitan area and emerging missions and infrastructure

Figure 6. Searching for *Spinanycta alabamensis* sp. nov. among and under mud-covered rocks at the type locality, Bobcat Cave, Madison County, Alabama, USA. Photograph by Amata Hinkle.

projects on Redstone Arsenal threaten cave life, including *Spinanycta alabamensis*, at all three cave systems (Bearden et al. 2022; USFWS 2022). At present, Bobcat and Muddy caves receives little human visitation; however, Rockhouse Cave receives a high level of unauthorized visitation despite its closed status, which has led to substantial vandalism and accumulation of trash in many sections of the cave.

Remarks. In many species of the subfamily Cubacubaninae, the area between the 3rd legs and the base of the abdomen has very distinct sexual secondary characters. In *Speleonycta ozarkensis*, the tibia of third leg has a large bulge with three distinctly long, sclerotized, and curved macrochaetae (Espinasa et al. 2014). *Anelpistina mexicana* (Espinasa, 1991) has on the lateral sides of urosternum III an extension with the shape of a hook or spine. Many species of *Anelpistina* have an articulated appendage in urosternum IV and *Prosthecina avita* Espinasa, 2000 has both the hook of urosternum III and the appendages in urosternum IV. Interestingly, the presence or absence of these characters follows little phylogenetic predictability, and sister species may lack

the character, but the character may be present in distantly related species. With the new species, a new sexual secondary character is described, the spine in urosternum I.

It appears that during reproduction, males in this group may use this part of the body. Evolution has developed a plethora of variable structures. All these structures include some type of chaeta. Sensory input during their interaction with females during courtship may be involved. Unfortunately, their reproductive behavior has yet to be described. Future research may resolve what is the function of all these intriguing structures.

Discussion

Before the description of *S. alabamensis*, 9 troglobiotic nicoletiids have been described from caves of Chihuahua and Coahuila in northern Mexico and the United States, including the states of Arkansas, Arizona, Oklahoma, and Texas. At least two undescribed species are known from caves of California. With the discovery of *S. alabamensis* in Alabama, the distribution of troglobiotic nicoletiids is extended into the Interior Low Plateau karst region in the southeastern United States. Additional diversity may be discovered in the future. Peck (1995) reported eyeless and unpigmented nicoletiids from three additional caves in northern Alabama: Cemetery Cave (ACS no. ADK444) in DeKalb County, Talley Ditch Cave (ACS no. AJK248) in Jackson County, and Shelta Cave (ACS no. AMD4) in Madison County. Unfortunately, the whereabouts of specimens from these caves are currently unknown. Shelta Cave is developed in the same geological formation as caves inhabited by *S. alabamensis* and is 13.4 km northwest of Bobcat Cave. Moreover, both caves, as well as Muddy and Rockhouse caves, share several troglobiotic and stygobiotic species (Cooper 1975; Peck 1989, 1995; Rheams et al. 1992; Niemiller et al. 2019; Niemiller unpubl. data). The nicoletiid from Shelta Cave may be *S. alabamensis* or a closely related species. The identity of nicoletiids reported from Cemetery and Talley Ditch caves is unclear. Cemetery Cave is located on the eastern escarpment of Sand Mountain, while Talley Ditch Cave is in the Crow Creek drainage on the eastern escarpment of the Cumberland Plateau. Both caves are located well east of the range of *S. alabamensis* in a different physiographic province. Thus, *S. alabamensis* is likely the first of several additional species of cave-dwelling nicoletiid to be formally described from karst regions of the southeastern United States.

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